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1: Cancer Res. 1998 May 1;58(9):1936-45.

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Isolation of human O6-alkylguanine-DNA alkyltransferase mutants highly resistant to inactivation by O6-benzylguanine.

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The activity of O6-alkylguanine-DNA alkyltransferase (AGT) protects cells from killing by methylating or chloroethylating agents. AGT is strongly inhibited by O6-benzylguanine (ED50, 0.2 microM), and this drug is presently undergoing clinical trials to enhance chemotherapy by alkylating agents. Point mutations such as P140A (ED50, 5 microM) render AGT resistant to O6-benzylguanine (BG). Selection for such mutants may prove to be a problem in the use of BG, and a better knowledge of the factors underlying resistance to BG will enable the rational design of improved inhibitors able to inactivate these mutants. BG-resistant AGT mutants may also be valuable for expression in bone marrow stem cells to reduce myelosuppression brought about by alkylating agents, to increase the therapeutic index of therapies including BG, and for use as a selectable marker to allow other genes to be expressed in such stem cells. We have therefore set up a general screen to obtain such mutants by using the ability of AGT to protect Escherichia coli GWR109 lacking endogenous AGT from killing by N-methyl-N'-nitro-N-nitrosoguanidine. When the cells were rendered permeable to BG by mutating the lipopolysaccharide membrane component forming strain TRG8, the protection by AGT expression was abolished by treating the cells with BG. The known P140A mutant was used to test the system and was highly selected for by treatment with 50 microM BG and 40 microg/ml N-methyl-N'-nitro-N-nitrosoguanidine. The sequence coding for PVP at positions 138-140 in AGT was replaced with a random nucleotide sequence, and this library was used to transform TRG8. All of the 59 colonies analyzed having AGT activity that survived the selection from the pool of 36,000 transformants were resistant to BG. Many (69%) of these mutants contained lysine at position 140, and all of these showed the highest level of resistance with <10% loss of activity when crude cell extracts were incubated with 1.2 mM BG. This result was

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confirmed with three mutants (P138K/V139L/P140K, P138M/V139L/P140K, and P140K), which were purified to homogeneity. The next most common residues found at position 140 were arginine (7%) and asparagine (7%). Studies carried out with purified preparations of mutants P140R and P140N revealed that these mutations also provided resistance to BG but to a lesser extent than P140K (ED50s of 190 and 7 microM, respectively). These results indicate that: (a) this screening method can be used to evaluate BG resistance of single or multiple changes throughout the AGT sequence; and (b) replacement of proline-140 with lysine is the most effective point mutation at this site causing BG resistance and is more than 200 times more effective than replacement with alanine.

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